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PATENT

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May 22, 2001
Date

M. A. Scarlett
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Martin A. Cheever and Mary L. Disis
Application No.	:	09/167,516
Filed	:	October 6, 1998
For	:	COMPOUNDS FOR ELICITING OR ENHANCING IMMUNE REACTIVITY TO HER-2/*neu* PROTEIN FOR PREVENTION OR TREATMENT OF MALIGNANCIES IN WHICH THE HER-2/*neu* ONCOGENE IS ASSOCIATED
Examiner	:	Karen A. Canella, Ph.D.
Art Unit	:	1642
Docket No.	:	920010.448C8
Date	:	May 22, 2001

Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner:

I, Martin A. Cheever, hereby declare as follows:

1. Mary L. Disis and I are coinventors of the subject matter described in the above-identified patent application (hereinafter referred to as "subject application").

2. I hold an M.D. and was employed at the time the subject application was filed by the University of Washington where I held the position of Professor of Medicine, Division of Medical Oncology. I am presently employed by Corixa Corporation of Seattle, Washington.

3. I have been involved in immunology for over 25 years and I have authored or coauthored about 100 publications in this field. My research specialty is immunology related to cancer.

4. I performed or supervised the performance of experiments as described in paragraphs 5 and 6 herein.

5. In vitro immunization involving recombinant Adenovirus infection of dendritic cells (DC) was demonstrated. An Adenovirus vector deleted for E1A and recombinant for the intracellular domain (ICD) of HER-2/*neu* protein was constructed and used to infect DC. Following maturation of the DC with CD40-L, priming cultures were initiated that contained 1.3 x 10^6 infected and matured DC and 1.8 x 10^7 PBMC. Cultures also included 10 ng/ml each IL-7 and IL-12, added at day 0, and 10 U/m1 IL-2, added at day 3. Prior to the second stimulation, $CD8^+$ cells were purified from the bulk culture using MACS columns, and $CD8^+$ cells were restimulated in 24 well plates with Adenovirus-ICD infected DC as antigen presenting cells (APC). The culture was stimulated twice more using autologous fibroblasts retrovirally transduced with ICD and tested for ICD-specific CTL activity by ^{51}Cr-release assay. As shown in **Figure 1**, the bulk line contained activity specific for ICD, since the line lysed autologous B-LCL infected with vaccinia-ICD but not vaccinia-EGFP or uninfected autologous B-LCL targets.

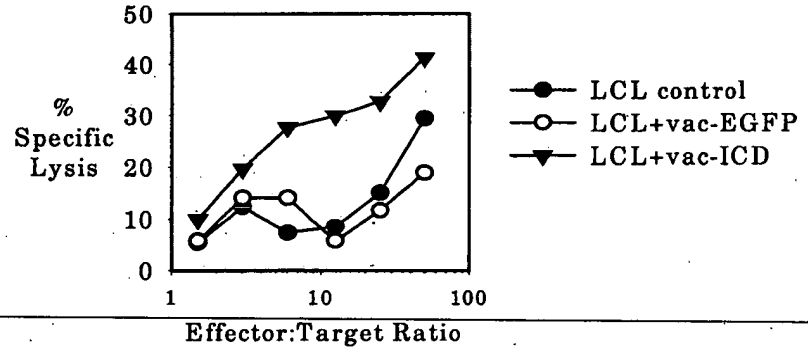


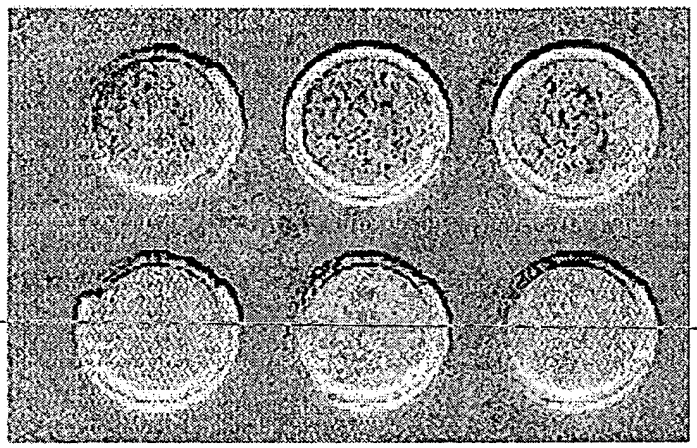
Figure 1: ^{51}Cr release assay demonstrating ICD-specific CTL activity in a T cell line generated by priming with DC infected with recombinant Adenovirus expressing ICD. Assay was a standard 4 hour ^{51}Cr release assay; targets were autologous B-LCL infected with recombinant vaccinia expressing ICD, EGFP, or uninfected. Each data point is the average of 3 measurements.

6. The ICD-specific line was then re-stimulated once on autologous LCL infected with vaccinia-ICD, and then split into two and stimulated either on autologous DC infected with Adenovirus-ICD or with anti-CD3; both of these sub-lines were tested for recognition of target cells that expressed ICD using an IFNγ ELISPOT assay. As shown in **Figure 2**, ICD-specific reactivity could be detected in both antigen (panel A) or anti-CD3 expanded (panel B) cultures. The average spot number from the triplicate wells was 344 on the ICD fibroblasts and 22 on the EGFP fibroblasts (antigen stimulation) and 365 (ICD) and 17 (EGFP) (anti-CD3 expansion).

(A)

ICD transduced fibroblasts

EGFP transduced fibroblasts



(B)

ICD transduced fibroblasts

EGFP transduced fibroblasts

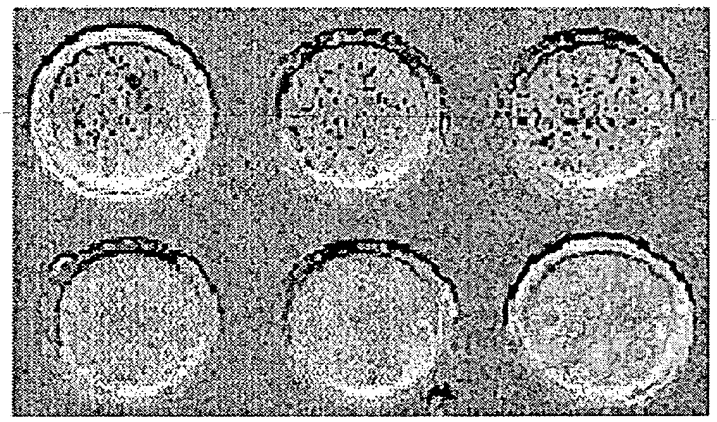


Figure 2: IFNγ ELISPOT analysis of $CD8^+$ T cell lines derived from in-vitro priming experiments using Adenovirus-ICD infected DC as APC. Data shown are in triplicate, from two sub-lines expanded for one cycle either on Adenovirus-ICD-infected DC (A) or anti-CD3 (B), tested on autologous fibroblasts transduced with ICD or EGFP. Fibroblasts were treated with IFNy 48-72 hours prior to the assay and washed to remove cytokine. 2×10^3 stimulators were plated per well with 2×10^4 responders for the original cell line and 4×10^4 responders for the expanded cell line.

7. Thus, immunization in vitro with recombinant adenovirus expressing ICD induces an immune response to HER-2/*neu* protein.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

Dated this 22nd day of May, 2001.

Martin A. Cheever

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